REMARKS

This is in response to the official action dated May 24, 2011. Reconsideration in view of the following is respectfully requested.

Claim Status

Claims 1, 3, 6, 8, 9, 12-15, 17-19 and 23-24 are pending in the application and stand rejected. Claims 2, 4-5, 7, 10-11, 16, and 20-23 were cancelled. Claims 1, 3, 6, 17, 18 and 24 have been amended.

Claim Rejections* - 35 *USC* § 112 *2nd paragraph/indefiniteness

Claims 1, 3, 6, 8, 9, 17-19 and 24

Improper Markush language and lack of antecedent basis have been corrected as requested by the Examiner. Terms have been changed for consistency which should address any clarity issues including the interrelationship of components or steps. The peptides are selected from the group consisting of, therefore polyclonal antibodies raised against one of the peptides suffices to induce polyclonal antibodies to be used in diagnosis.

Claim 23 –no further limitation

Claim 23 is cancelled.

Claim 18- recital of immunological test kit

"The" immunological test kit is recited as requested by the Examiner.

Claim Rejections - 35 U.S.C. § 102 Novelty in view of Sziegolet or Scheefers

The present claims are directed to antibodies "raised against … synthetic peptides … wherein polyclonal antibodies that react specifically with a human pancreatic elastase iso-enzyme in stool or body fluids are induced by immunization of a vertebrate animal administering one or more of said synthetic peptides".

Notably, when antibodies are raised by immunizing a vertebrate organism, a mixture of different antibodies is formed. The antibody mixture differs from the antibody mixture formed when immunizing with purified enzyme or randomly selected fragments thereof. It is statistically impossible for the two mixtures to be identical. For example, the claimed antibodies do **not** recognize Thr-Met-Val-Ala-Gly-Gly-Asp-Ile-Arg (SEQ ID NO: 1), which is the epitope identified and described in the Scheefers patent.

Significantly, the claims do not cover the "the whole peptide(s) as comprised in purified enzume iso-forms"/"purified enzyme isoform(s) as asserted by the Examiner. In contrast, the claims include antibodies formed through immunization with specific peptides, which **consists of** the synthetic peptides recited, and results in antibodies raised specifically against these synthetic peptides, which are separated and purified. These synthetic peptide-based antibodies are then used to detect human pancreatic elastase iso-enzyme. The prior art does not disclose any method wherein antibodies are raised against one or more of the recited peptides.

Contrary to what the Examiner appears to assert, none of the references suggests that any enzyme preparation, even if fragmented, would inherently comprise the peptides recited in the present claims. It is not even clear if a random fragmentation of elastase would contain **any** fragments identical to the synthesized peptides used in the invention. Some parts of a protein break more easily than others. For the sequence of the synthetic peptides to occur randomly by fragmentation, both ends would have to break at the same time while the amino acids in the middle must stay intact. Even if this did occur, for it to have a similar effect as synthetic peptides it would have to occur to a high percentage. Even if by chance, by random fragmentation, the elastase protein would break in just the right locations and forms peptides identical in both length and sequence to the synthetic peptides used to immunize animals and form antibodies in the present invention, and even if this did occur to a sufficient degree to provide sufficient sensitivity, this mixture of peptides would include other fragments and would not raise antibodies with the same characteristics, the result would be less reproducible, and would certainly contain cross-reacting antibodies or antibodies not specific to elastase proteins/particular elastase isoforms.

This is very likely the reason why Scheefers does not use a mix of random peptide fragments. Instead, Scheefers and Sziegolet use the highly purified whole elastase enzyme, or (in case of Scheefers) the one specific peptide epitope Scheefers surprisingly identified to be sensitive and selective.

Significantly, the present claims do not cover the whole elastase protein/enzyme nor Scheefers' peptide epitope.

Notably, the generic term "fragments (of elastase)" in the prior art does not disclose any specific peptide, nor does it disclose the synthetic peptides employed in the present invention, which are characterized by specificity/lack of cross-reactions and sufficient binding and signal strength that are useful to immunize animals and form the resulting antibodies which are suitable to detect human elastase.

To reproducibly achieve antibodies that are specific for elastase and diagnostically relevant, peptides have to be selected from a multitude of possible fragments. There is no disclosure nor even guidance in the prior art that helps identifying and selecting other peptides that may have the required characteristics.

Accordingly, instant claims reciting specific elastase peptides are novel.

Claim Rejections - 35 U.S.C. § 103 Obviousness

The Examiner asserts it was conventional in the art to elicit antibodies to peptides derived from a known sequence for [diagnostic] use as disclosed in Scheefers, that this selection was routine in the art and within the skill of an ordinary practitioner from the sequence comparisons presented in Tani et al., and concludes that there was motivation to combine references to arrive at the present invention with an "extremely reasonable expectation of success". Furthermore the Examiner asserts there was motivation to select dissimilar sequences to elicit antibodies specific for the isoforms.

However, merely selecting dissimilar peptides among elastase isoforms to elicit isoform-specific antibodies, as the examiner asserts, does not ensure general specificity/selectivity for the protein to be detected, nor a lack of cross-reactivity with other unrelated proteins, which is a problem especially for synthetic peptide based antibodies.

Notably, there is no teaching anywhere in the prior art, as to which regions of the elastase protein to select and which to avoid to prevent e.g. cross-reactions with other isoforms, pig elastase, and a multitude of other proteins (which need not necessarily be related in case of synthetic peptides, adding to the unpredictability) present in body fluids, while maintaining sensitivity.

Not only is it unclear which regions of these proteins provide sensitivity or specificity, it is also unclear which other proteins may cross-react with the recited peptides. Using such peptides bears different risks for cross-reactiveness than using the whole protein or a mixture of various/all fragments thereof. As is well known in the art, these risks are very much unpredictable, and accordingly far from obvious, as demonstrated for example by the excitatory amino acid transporter 3 (EAAT3), to give just one example, see attached abstract (Exhibit A) on the specificity of antibodies (Holmseth et al., Neuroscience. 2005;136(3):649-60). Of the 13 antibodies synthesized by Holmseth, "**most"** failed to recognize the native protein, and others recognized other proteins, some completely unrelated in sequence. When a peptide is used for immunization rather than the native protein, its three-dimensional structure usually is very different. However, this three-dimensional structure is crucial for antibody binding in immunization.

As the abstract points out, it is easy to raise antibodies, however, it is very much unpredictable if they will recognize the desired target protein (here **the majority** did not), and if the target is in fact recognized, second and third points of unpredictability are whether any other protein not targeted is recognized at the same time, i.e. whether the antibody is specific and recognizes the target only, and whether the sensitivity of target recognition is high enough and any cross-reactivity low enough to allow for a test that can reliably be used for clinical

diagnostics. For example, in the abstract a number of peptides recognized a completely unrelated abundant protein (tubulin).

All of this shows that the provision of specific antibodies recognizing a particular protein from synthetic peptides has a high degree of unpredictability and no reasonable expectation of success, in contrast to the Examiner's assertion.

The same is shown in the dissertation of Dr. Kleinert on the development of a laboratory method for detection of human elastase in stool samples as a marker for exocrine pancreas insufficiency, which is submitted separately with this response (Rainer Kleinert, Entwicklung einer Labormethode zum Nachweis der humane pankreatischen Elastase in faekalen Proben als Marker fuer die Diagnose einer exokrinen Pankreasinsuffizienz, Greifswald 2001). Relevant excerpts translated into English are discussed below.

On page 28 under item 2.3.1 of Rainer Kleinert's dissertation, examination of specificity of the antibodies raised against the peptide antigen, the scientist expresses surprise that " These great results were unexpected, since it was known to us from experiments with peptides of other proteins, that the antibody production on the basis of relatively short peptides as antigens in animals is extremely difficult".

Turning to the prior art disclosures, to shortly reiterate, Scheefers discloses a particular **different** synthetic elastase peptide epitope, or fragments **of this epitope**, and an assay used in serum and stool to detect it. Examples in Scheefers use highly purified enzyme **whole**, or the epitope. Scheefer smentions that antibodies could be raised with fragments of the highly purified enzyme. Naturally the majority can be expected to either cross-react with non-target proteins or have a sensitivity that is too low. There is no guidance which fragments of the enzyme to choose and avoid cross-reactivity apart from the one specific epitope disclosed. Significantly, the Scheefers patent granted with claims to the particular peptide epitope only, which was used to raise antibodies that were selective and did not cross-react. Scheefer spoints at the unpredictability of selectively detecting elastase: "**Surprisingly**, it was found that antibodies

directed against **this epitope** of human elastase 1 selectively recognize the marker enzyme and thereby discriminate against other antigens. " See excerpt from Scheefer below.

> According to this invention, this object is achieved by means of an antibody directed against the epitope having the amino acid sequence Thr-Met-Val-Ala-Gly-Gly-Asp-Ile-Arg (SEQ ID NO:1). Surprisingly, it was found that antibodies directed against this epitope of human elastase 1 selectively recognize the marker enzyme and thereby discriminate against other antigens.

Tani discloses elastase III. Harlow relates to synthetic peptide design to raise antibodies and discloses that "there is no one correct answer" to" what sequence should be used for the immunogen", that while "suggestions for peptide choices" can be made, "preparing anti-peptide antibodies is still an empirical exercise", and "what works well for one immunogen may fail completely for another" (Harlow, page 75, 1st paragraph).

None of the prior art disclosures alone or in combination teaches the synthetic peptides selected in the present invention which allow the formation of antibodies that will regognize the elastase target while avoiding cross-reactivity with other proteins in body fluids.

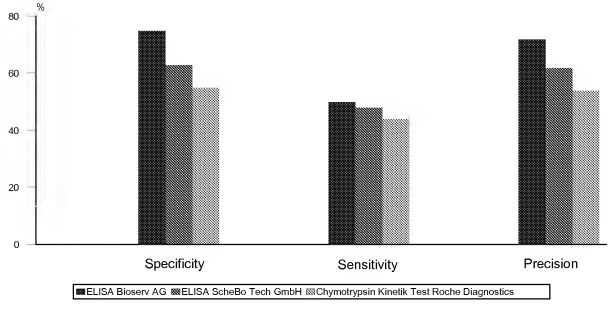
Nothing more is required for patentability. The novel alternative method is at least comparable to existing different methods and a useful alternative diagnostic tool for doctors and their patients. It is noted that the Examiner admitted comparability (as well as a "slight improvement") in view of the submitted abstracts in the last communication.

Moreover, the invention has certain advantages over the prior art, as discussed in detail in the previous response and as shown in the abstracts previously submitted.

In addition, Dr. Kleinert's dissertation (Rainer Kleinert, Entwicklung einer Labormethode zum Nachweis der humane pankreatischen Elastase in faekalen Proben als Marker fuer die Diagnose einer exokrinen Pankreasinsuffizienz, Greifswald 2001) demonstrate these advantages as well. For example, on page 34-35 in figure 3, it can be seen in a Western blot that not only monomeric whole elastase (28 kD) but also its dimeric form of about 50 kD as well as a smaller

proteolytic fragment of elastase of about 10 kD, both of which were found in stool samples, are recognized by antibodies K10 and K12, which are antibodies according to the invention. This fact allows for selectivity and a higher sensitivity as demonstrated e.g. in the previously submitted abstracts and in the dissertation of Dr. Kleinert, for example in a comparison to two commercial test systems, see figure 23.

Figure 23 on page 78 of Dr. Kleinert's dissertation compares the antibodies claimed ("ELISA Bioserv AG") to a monoclonal antibody product (ELISA ScheBo Tech GmbH) and to a different test system based on chymotrypsin (Chymotrypsin Kinetik Test Roche Diagnostics). The results shown in figure 23 demonstrate that the test based on the claimed antibodies favorably compares to both test methods in specificity, sensitivity and precision, as discussed on pages 77-78: "Comparative analysis of two commercially available indirect test systems for the diagnosis of exocrine pancreas insufficiency and the herein developed diagnostic method was carried out using 699 stool samples. The specificity and sensitivity, respectively, of our method was 12 or 2% higher, respectively, than the commercially available elastase 1 tests, and 20% or 6% higher, respectively, than for the chymotrypsin kinetic test. The resulting precision for our elastase 1 diagnostic method was 72%, for the commercially available elastase 1 ELISA it was 62%, and for the enzyme kinetic test it was 54%. The results are shown graphically in figure 23."



The applicant again stresses that the claims are directed to "…antibodies raised against one or more synthetic peptide selected from the group **consisting of**…" and, contrary to the Examiner's assertion do not cover the purified enzyme or random fragments thereof disclosed or suggested in the prior art, nor the single specific peptide disclosed by Scheefers.

The Examiner asserts that the shown "slight difference" is not commensurate in scope with the invention as claimed, and instead teaches the comparability of the assay with the prior art, dismissing the improvements with the remark that "showing of a difference in degree is not evidence of a difference in kind."

However, this is beside the point. The provision of an alternative novel and unobvious method is a valid patentable contribution in itself.

The present invention furthermore does provide an improvement, which even if considered "slight" by the Examiner, is currently enjoyed by medical practitioners performing the novel diagnostic test, and not least by their patients, and more significantly relates to a novel and unobvious method.

For the reasons detailed above, applicant believes the invention is unobvious.

Wherefore, allowance of all claims is earnestly solicited.

Respectfully submitted,

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